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THE SMALL HEAT-SHOCK PROTEIN HSP27 SHOWS DECREASED EXPRESSION IN OA-CHONDROCYTES AND MEDIATES IL-6 SECRETION IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: HSP27 (HSPb1) is one of the small heat-shock proteins (sHSP), a protein family characterized by a molecular weight below 30 kDa. Recently, we reported the differential expression of alphaBcrystallin, another sHSP (Lambrecht et al, Arthritis Rheum, 2009). Based on the functional and structural relationship between alphaBcrystallin and HSP27, we further performed differential expression analysis on HSP27. We aimed to achieve further insights in the involvement of small heat-shock proteins, more specific HSP27, in the biology of chondrocytes.
Methods: Western blot and real-time RT-PCR analysis were performed to determine the expression levels of HSP27 in healthy and OA chondrocytes cultured in alginate beads. RNA-interference mediated gene knock-down was used to explore the role of this small heat shock protein in IL-1b activated pathways by transfecting low concentrations of siRNA in cultured chondrocytes. Upon knock-down of HSP27, cells were stimulated by IL-1b and IL-6 concentrations were determined by ELISA.
Results: Western blot of healthy and OA chondrocyte lysates showed a decreased abundance of HSP27 in OA. Moreover, real-time RT-PCR confirmed the differential expression at the mRNA-level between chondrocytes isolated from visually intact and visually damaged zones of OA cartilage. The pro-inflammatory cytokines IL-1beta and TNF-alpha, both down regulated HP27 expression. Transfection of low concentrations siRNA in cultured chondrocytes resulted in an efficient knock down of HSP27 gene expression. This decreased HSP27 expression results in a reduced secretion of IL-6, in response to IL-1b.[[Unsupported Character - ]]
Conclusions: This study adds to the evidence that small heat-shock proteins may be important mediators in chondrocyte biology during the development of OA. Our study showed the involvement of HSP27 in IL-1b induced IL-6 secretion in human articular chondrocytes.

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CALCIFICATIONS IN OSTEOARTHRITIC HUMAN ARTICULAR CARTILAGE: ASSESSMENT OF CALCIUM COMPOUNDS THROUGH AN *EX VIVO* INVESTIGATION BASED ON XANES SPECTROSCOPY USING SYNCHROTRON RADIATION

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Purpose: Calcium (Ca)-containing crystals (CCs), including basic calcium phosphate crystals and calcium pyrophosphate crystals are associated with severe forms of osteoarthritis (OA). Recent reports strongly support that mineralization of articular cartilage is an indissociable process of OA. The imbalance between extracellular matrix components can promote cartilage mineralization. However, the role of Ca compounds in this process is poorly understood. Our aim was to assess the presence and the crystallinity of Ca phosphate phases between areas with or without calcifications in human OA articular cartilage.
Methods: Six patients (mean age 74±9 years), who underwent total knee joint replacement for primary OA, were randomly included. *Ex vivo* specimen included femoral condyle and tibial plateau cartilages, from both medial and lateral compartments. Clinical data and preoperative knee X-rays were obtained. For each femoro-tibial compartment, 6 samples were collected, consisting in 1-mm-thick slices, cut either tangentially or perpendicularly to the articular surface. CCs presence and biochemical composition was assessed using Fourier transform infrared spectroscopy. Next, Ca compounds composition and distribution was assessed using X-ray absorption spectroscopy (XAS) synchrotron radiation, a large scale instrument, at the Ca *K*-absorption edge.
Results: Overall, 12 samples were analysed using both techniques. Ca compound forms differed between areas with or without calcifications. In non-calcified areas, tissue non diffusible Ca displayed specific XAS spectra, compared to calcified areas, in which each crystal type had a particular XAS fingerprint. Strikingly, when cartilage was calcified over than 15%, the percentage of tissue non diffusible Ca dramatically decreased, with Ca compounds mainly involved in the calcifications.
Conclusions: We provide *ex vivo* direct structural evidence at the atomic scale of Ca phosphate phases differences between human OA cartilage areas with or without CCs. In calcified areas, Ca compounds are mainly involved in the calcifications, with CCs Ca phosphate phase becoming the predominant detectable Ca phase.

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THE NUCLEAR ORPHAN RECEPTOR NR4A3 IS REQUIRED FOR MMP-13 AND ADAMTS-5 EXPRESSION IN HUMAN CHONDROCYTES

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Purpose: In osteoarthritis (OA) gene expression of chondrocytes is deregulated towards catabolism, leading to progressive cartilage matrix degradation. The mechanisms responsible for this imbalance are not fully understood.
Nuclear orphan receptors act as ligand-independent transcriptionally activated regulator proteins. In a global analysis of nuclear receptor expression in OA cartilage by Collins-Racie et.al. the nuclear orphan receptor NR4A3 was found to be highly deregulated. As its function in chondrocytes is still unknown, we evaluated the influence of NR4A3 on chondrocyte gene expression.
Methods: Human cartilage specimens were obtained from patients undergoing total knee joint replacement. Chondrocytes were isolated using collagenase B. Primary human chondrocytes and the human chondrocyte cell-line C28I2 were grown in monolayer and cultured in Ham's F-12/DMEM (1:1) and 10% FCS. Expression of NR4A3 was evaluated by reverse-transcriptase PCR. For siRNA knock-down of NR4A3 cells were transfected with specific anti-sense oligonucleotides using lipofectamine. After 24h gene expression of ADAMTS-4 and -5, MMP-13, iNOS, aggrecan and pro-collagen II, was investigated using real-time PCR.
Results: NR4A3 mRNA was found to be expressed in OA chondrocytes and the C28I2 cell-line. siRNA knock-down achieved 60% (± 4%) reduction of NR4A3 mRNA expression. The decrease in NR4A3 expression led to a decline of MMP-13 expression by 52% (± 9%). Furthermore ADAMTS-5 mRNA expression was reduced by 50% (± 3%). Gene expression of ADAMTS-4, iNOS, aggrecan and pro-collagen II remained unaltered in the presence of diminished NR4A3 expression.
Conclusions: We confirmed the expression of the nuclear orphan receptor NR4A3 in OA chondrocytes and C28I2 cells. Furthermore its presence is required for maintenance of MMP-13 and ADAMTS-5 expression. Therefore NR4A3 could be a novel promising therapeutic target in OA chondrocytes.

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OPTIMIZED ALKYLATED CYCLODEXTRIN POLYSULPHATES RESTORE OSTEOARTHRITIC CHONDROCYTE EXTRACELLULAR MATRIX METABOLISM

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Purpose: To compare the ability of different cyclodextrin polysulphate derivatives to affect human articular cartilage cell metabolism *in vitro* and to act as Disease Modifying OsteoArthritis Drugs *in vivo*.
Methods: OA chondrocytes were cultured in gelled alginate and exposed to 5 μg/ml of 2,3,6-tri-O-methyl-β-cyclodextrin (ME-CD), 2,3-di-O-methyl-6-sulphate-β-cyclodextrin (ME-CD-6-S), 2,6-di-O-methyl-3-sulphate-β-cyclodextrin (ME-CD-3-S), (2-carboxyethyl)-β-cyclodextrin polysulphate (CE-CDPS), (2-hydroxypropyl)-β-cyclodextrin polysulphate (HP-CDPS), 6-monoamino-6-monodeoxy-β-cyclodextrin polysulphate (MA-CDPS) or β-cyclodextrin polysulphate (CDPS) during 5 days. Effects on IL-1-driven chondrocyte extracellular matrix (ECM) metabolism was assayed by analysis of the accumulation of aggrecan in the interterritorial matrix and by the release of IL-6 in the culture supernatant. MA-CDPS,